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Evaluation of TECRA Broth, Bolton Broth, and Direct Plating for Recovery of *Campylobacter* spp. from Broiler Carcass Rinsates from Commercial Processing Plants†

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ABSTRACT

The purpose of this study was to compare a conventional culture broth method (Bolton enrichment), a newly developed proprietary broth method (TECRA *Campylobacter* enrichment), and direct plating for recovery of *Campylobacter* spp. from chicken carcass rinsates. Whole carcass rinses were taken from 140 carcasses at rehang (immediately after defeathering but before evisceration) and from 140 carcasses at postchill from eight different processing plants in the United States. The rinsate samples were packed in ice and shipped overnight to the laboratory. Aliquots of the rinsate were transferred into Bolton and TECRA enrichment broths and were direct plated. Standard laboratory procedures with Campy-cefex plates were followed for recovery of *Campylobacter* spp. For rehang carcasses, 94% were positive for *Campylobacter* spp. with the TECRA enrichment broth and 74% were positive with the Bolton enrichment broth. For postchill carcasses, 74% were positive for *Campylobacter* spp. with the TECRA enrichment broth and 71% were positive with the Bolton enrichment broth. Compared with the Bolton enrichment broth, TECRA enrichment broth significantly suppressed non-*Campylobacter* microflora ($P < 0.05$). Overall, TECRA enrichment broth yielded an 11% higher total number of *Campylobacter*-positive samples compared with the Bolton enrichment broth. *Campylobacter* spp. detection in postchill samples was significantly greater ($P < 0.05$) by enrichment (84%) than by direct plating (19%). The high number of *Campylobacter*-positive samples obtained with all procedures indicated that 99% of the carcass rinsates obtained at rehang and 84% obtained at postchill contained *Campylobacter* spp.

The genus *Campylobacter* has been the subject of increased interest as a possible threat to food safety because of the rise in human enteritis cases associated with the consumption or handling of foods contaminated with this organism. The infectious dose for *Campylobacter* in humans can be as low as a few hundred cells *(10, 17). Campylobacter* spp. can be affected by environmental conditions outside of an animal's alimentary tract *(31),* such as drying, low atmospheric oxygen concentration, and low pH (less than or equal to 4.7), but are still a leading cause of bacterial gastroenteritis in humans. In the United States, approximately 2.5 million people are infected each year via consumption of foods containing *Campylobacter (30).* Four *Campylobacter* species, *C. jejuni, C. coli, C. lari,* and *C. upsaliensis,* are thermophilic and clinically important because they are dominant causative agents of human campylobacteriosis *(11, 22, 25). C. jejuni* most often is the primary cause of bacterial gastroenteritis in the United States and in many other developed countries, with *C. coli* second *(28).* In the United States, *Campylobacter* and *Salmonella* alternate as the leading bacteria associated with foodborne illness *(1, 30).*

Cross-contamination of food products is a major contributor to human illness. Outbreaks of human campylobacteriosis have been associated with raw milk, untreated water, and raw poultry meat. Poultry carcasses are frequently contaminated with this organism and may be responsible for approximately 70% of sporadic campylobacteriosis cases *(16, 44).* Contamination is thought to originate from the intestinal tract of poultry and spreads to the carcass during transport and processing *(6, 8).* The crops of broiler chickens, particularly after feed withdrawal before transport to the processing facility, may harbor large numbers of *Campylobacter* cells *(6, 20, 42). Campylobacter* levels in the intestinal tract of broilers entering the processing plant can be 10^7 CFU/g of cecal contents, and when whole carcasses with feathers are rinsed 10^6 CFU/ml of rinse can be recovered from the carcass exterior *(8, 33).*

Campylobacter spp. are fastidious organisms whose culture requires a particular growth temperature and gaseous environment and nutrient-rich basal medium. Current conventional methods for detecting *Campylobacter* spp. in samples involve either selective enrichment followed by plating or direct plating onto selective media, microscopic observation, and biochemical confirmation or confirmation

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through latex agglutination. Numerous growth media for *Campylobacter* spp. have been developed and evaluated: Skirrow formulation, Preston, semisolid blood-free selective medium, charcoal cefoperazone deoxycholate agar, Campy-*Brucella* agar, Abeyta-Hunt-Bark agar, Campy-Line, and Campy-cefex *(18, 29, 35, 39, 41)*. Membrane filtration techniques and semisolid enrichment media also have been developed for recovery of *Campylobacter* spp. *(13, 23, 40)*.

Selective enrichment broths are used to improve the recovery of *Campylobacter* cells that may be in low numbers, injured, or stressed *(21, 43)*. Enrichment broths differ in their nutrient composition, oxygen-quenching agents, incubation time, temperature, and environment, and antibiotics used to suppress growth of competing organisms. Examples of selective enrichment broths that have been evaluated are Preston broth, Bolton broth, *Campylobacter* enrichment broth, blood-free enrichment broth, buffered peptone water, and Hunt enrichment broth *(4, 9, 34)*. Use of a selective enrichment broth for recovery of *Campylobacter* spp. may increase detection sensitivity compared with that possible with direct plating as long as competitive organisms can be suppressed *(21, 36)*. An investigation of the efficacy of enrichment for isolation of *C. jejuni* from water and foods revealed that the number of heat- or freeze-damaged cells recovered was increased by enrichment in basal or selective medium *(21)*.

A newly developed *Campylobacter* enrichment broth (TECRA broth) may allow for improved recovery of *Campylobacter* spp. compared with that obtained with other enrichment broths *(2)*. The TECRA *Campylobacter* visual immunoassay involves aerobic enrichment in a proprietary TECRA *Campylobacter* enrichment broth for 48 h at 42°C followed by a 6-h enzyme-linked immunosorbent assay (ELISA). This method was compared with a conventional cultural method that involved enrichment in Bolton medium (containing lysed horse blood) and then plating on Campy-cefex agar. Significantly more samples ($P < 0.05$) were positive for *Campylobacter* spp. with the ELISA than with the conventional cultural method *(2)*. The increased recovery may have been the result of differences between the two enrichment broths.

The objectives of the current study were threefold: (i) compare TECRA broth to Bolton broth for the recovery of natural levels of *Campylobacter* spp. from carcass rinse samples obtained at rehang and postchill in commercial processing facilities, (ii) determine the efficacy of TECRA and Bolton broths for reducing background microflora during incubation, and (iii) compare enrichment methods and direct plating for recovery of *Campylobacter* spp. from carcass rinse samples.

MATERIALS AND METHODS

Sampling procedures. Samples were collected from eight processing facilities throughout the United States. A total of 140 rehang carcasses (after defeathering but before evisceration) and 140 postchill carcasses were used in the study. From each of three processing facilities, 10 rehang carcasses and 10 postchill carcasses were obtained; from each of four processing facilities, 20 rehang carcasses and 20 postchill carcasses were obtained; and from one processing facility, 30 rehang carcasses and 30 postchill carcasses were obtained. Each carcass was placed in a sterile plastic bag, and 100 ml of sterile water was added for rinsing *(14)*. Carcass rinsates were poured into sterile specimen cups, placed on ice, and transported overnight to the laboratory, where samples were analyzed.

Enrichment broth procedures. The efficacy of TECRA enrichment broth for recovery of *Campylobacter* spp. from broiler carcass rinses was compared with that of Bolton enrichment broth with lysed horse blood, and these enrichment procedures were compared with direct plating. The plating medium used for all procedures was Campy-cefex agar. From each of the rinse samples, 5 ml was transferred into sterile sampling bags containing 45 ml of Bolton enrichment broth (Acumedia Manufacturers, Inc., Baltimore, MD) containing lysed horse blood (Lampire Biological, Everett, PA) and an antibiotic supplement (Dalynn Biologicals, Calgary, AB, Canada). Another 5 ml was transferred to bags of TECRA enrichment broth (TECRA Int. Pty. Ltd., Frenchs Forest, New South Wales, Australia). The Bolton broth samples were incubated at 42°C for 48 h in a microaerobic environment of 5% O_2, 10% CO_2, and 85% N_2 (Airgas, Athens, GA). The TECRA broth samples were incubated for 48 h at 42°C in an aerobic environment as specified in the TECRA CAMVIA protocol *(2)*. After incubation, 0.1-ml aliquots of the Bolton and TECRA broth cultures was streaked onto Campy-cefex agar plates (Acumedia). The plates were then incubated in a microaerobic environment for 48 h at 42°C. Presence of *Campylobacter* colonies on the plates from all samples was confirmed using phase-contrast microscopy and observing the characteristic rapidly darting cells in wet mounts. For further confirmation, latex slide agglutination (Microgen Bioproducts Limited, Camberley, Surrey, UK) was evaluated.

Direct plating procedure. A 0.25-ml aliquot of the sample rinsate was spread plated onto each of four Campy-cefex plates undiluted to maximize the detection level (1 CFU/ml). Serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) of rehang and postchill rinse samples were prepared using phosphate-buffered saline. A 0.1-ml aliquot from the dilutions was plated in duplicate onto Campy-cefex agar plates. The plates were then incubated in a microaerobic environment at 42°C for 48 h, and presumptive *Campylobacter* colonies were confirmed as described for the enrichment samples.

Background microflora evaluation. A technique based on ecometric principles was used to measure background microflora *(3, 27, 32)*. The ecometric technique was modified, and a four-quadrant streak technique was used. Samples from five processing facilities were used to estimate enrichment broth efficacy for suppressing non-*Campylobacter* microflora during broth incubation by evaluating non-*Campylobacter* colonies on Campy-cefex plates. The method involved streaking aliquots from the enrichment broth cultures onto quadrants of the plate. The initial aliquot (0.1 ml) was streaked onto the plating medium in quadrant 1. Material from that quadrant was then picked up with a sterile loop and streaked onto quadrant 2. This process was repeated for quadrants 3 and 4. After incubation, the growth of non-*Campylobacter* colonies on Campy-cefex plates was expressed as the absolute growth index (AGI). Growth on all four quadrants was given an AGI of 4, growth on quadrants 1 through 3 was given an AGI of 3, etc. Higher AGI values indicated a higher number of non-*Campylobacter* colonies originating from the incubated enrichment broth. The numbers obtained from the samples enriched in Bolton

TABLE 1. Campylobacter *spp. prevalence evaluated by TECRA broth, Bolton broth, and direct plating of rehang carcass rinse samples obtained from eight poultry processing facilities*

Facility no.	Prevalence[a]		
	TECRA	Bolton	Direct plating
1	10/10 A	1/10 B	0/10 B
2	10/10 A	10/10 A	10/10 A
3	9/10 A	10/10 A	10/10 A
4	20/20 A	15/20 A	20/20 A
5	26/30 A	21/30 B	30/30 A
6	16/20 A	16/20 A	18/20 A
7	20/20 A	20/20 A	20/20 A
8	20/20 A	10/20 B	4/20 C
Total	131/140 A	103/140 B	112/140 B

[a] Values are the number of positive carcasses/number of carcasses tested. Within each row, prevalences with different letters are significantly different ($P < 0.05$, χ^2 test for independence).

broth and TECRA broth were compared allowing for a semiquantitative estimation of the ability of each broth to suppress non-*Campylobacter* microflora. The identity of recurring non-*Campylobacter* colony types was determined. Each isolate was transferred onto total plate count agar and incubated for 24 h at 37°C. An isolated colony was obtained from the plate and identified by biochemical testing with the Vitex 2 system (bioMérieux, Inc., Durham, NC).

Statistical analysis. The chi-square test for independence was used to evaluate differences in the total number of carcasses determined to be positive for *Campylobacter* by the TECRA broth enrichment method, the Bolton broth enrichment method, or direct plating. The Kruskal-Wallis test and Dunn's multiple comparisons test were used to evaluate the differences in suppression of non-*Campylobacter* colonies by the enrichment broths *(15)*. For all tests, significance was set at a P value of less than 0.05. All statistical analyses were conducted using InStat 3.0 (GraphPad Software, Inc., San Diego, CA).

RESULTS

The incidence of *Campylobacter* spp. in each processing plant was determined by direct plating and plating after enrichment in TECRA and Bolton broths (Tables 1 and 2). The overall prevalences of *Campylobacter* spp. in the carcass rinses taken at rehang as determined by enrichment in TECRA broth (Table 1) were significantly different ($P < 0.05$) than those obtained by enrichment in Bolton broth and by direct plating. The overall prevalences of *Campylobacter* spp. in carcass rinsates taken at postchill as determined by the TECRA and Bolton enrichment methods (Table 2) were not significantly different ($P > 0.05$). Significantly fewer carcasses overall ($P < 0.05$) were determined to be *Campylobacter* spp. positive by direct plating compared with enrichment of the postchill samples. Evaluation of *Campylobacter* prevalence rates by combining the different enrichment methods are shown in Tables 3 and 4. The combination of the two enrichment broth methods had the greatest overall sensitivity for *Campylobacter* spp. in the rinsates.

The average number of quadrants on Campy-cefex

TABLE 2. Campylobacter *spp. prevalence evaluated by TECRA broth, Bolton broth, and direct plating of postchill carcass rinse samples obtained from eight poultry processing facilities*

Facility no.	Prevalence[a]		
	TECRA	Bolton	Direct plating
1	9/10 A	6/10 A	0/10 B
2	6/10 A	9/10 A	0/10 B
3	10/10 A	10/10 A	0/10 B
4	20/20 A	18/20 A	16/20 A
5	24/30 A	19/30 A	4/30 B
6	0/20 B	6/20 B	6/20 B
7	16/20 A	16/20 A	0/20 B
8	19/20 A	16/20 A	0/20 B
Total	104/140 A	100/140 A	26/140 B

[a] Values are the number of positive carcasses/number of carcasses tested. Within each row, prevalences with different letters are significantly different ($P < 0.05$, χ^2 test for independence).

TABLE 3. Campylobacter *spp. prevalence evaluated by combinations of TECRA broth, Bolton broth, and direct plating methods used with rehang carcass rinse samples*

Facility no.	Prevalence[a]			
	T, B, D	T, B	T, D	B, D
1	10/10 A	10/10 A	10/10 A	1/10 B
2	10/10 A	10/10 A	10/10 A	10/10 A
3	10/10 A	10/10 A	10/10 A	10/10 A
4	20/20 A	20/20 A	20/20 A	20/20 A
5	30/30 A	29/30 A	30/30 A	30/30 A
6	19/20 A	19/20 A	19/20 A	19/20 A
7	20/20 A	20/20 A	20/20 A	20/20 A
8	20/20 A	20/20 A	20/20 A	12/20 B
Total	139/140 A	138/140 A	139/140 A	122/140 B

[a] T, TECRA; B, Bolton; D, direct plating. Values are the number of positive carcasses/number of carcasses tested. Within each row, prevalences with different letters are significantly different ($P < 0.05$, χ^2 test for independence).

TABLE 4. Campylobacter *spp. prevalence evaluated by combinations of TECRA broth, Bolton broth, and direct plating methods used with postchill carcass rinse samples*

Facility no.	Prevalence[a]			
	T, B, D	T, B	T, D	B, D
1	10/10 A	10/10 A	9/10 A	6/10 B
2	10/10 A	10/10 A	6/10 A	9/10 A
3	10/10 A	10/10 A	10/10 A	10/10 A
4	20/20 A	20/20 A	20/20 A	18/20 A
5	24/30 A	24/30 A	24/30 A	19/30 A
6	7/20 A	6/20 A	6/20 A	6/20 A
7	17/20 A	17/20 A	16/20 A	16/20 A
8	20/20 A	20/20 A	19/20 A	16/20 A
Total	118/140 A	117/140 A	110/140 AB	100/140 B

[a] T, TECRA; B, Bolton; D, direct plating. Values are the number of positive carcasses/number of carcasses tested. Within each row, prevalences with different letters are significantly different ($P < 0.05$, χ^2 test for independence).

TABLE 5. *Mean absolute growth index of non*-Campylobacter *colonies present on Campy-cefex agar plates after streaking from TECRA and Bolton broth cultures of rehang and postchill samples from five poultry processing facilities*

Facility no.	Rehang samples		Postchill samples	
	Bolton	TECRA	Bolton	TECRA
1	4.0	2.7	3.6	0.9
2	3.0	0.0	2.7	0.7
3	2.7	1.0	0.7	0.2
5	3.8	2.0	1.0	0.3
7	3.5	2.4	1.0	0.2
Mean ± SE[a]	3.47 ± 0.11 A	1.09 ± 0.12 B	1.49 ± 0.16 B	0.39 ± 0.07 C

[a] Mean ± standard error of all individual samples within each of the five processing facilities. Values with different letters are significantly different ($P < 0.05$, χ^2 test for independence).

agar plates containing non-*Campylobacter* colonies after streaking from the two enrichment broth cultures from five of the processing plants is shown in Table 5. Overall, significantly fewer quadrants in plates streaked with the TECRA enrichment broth cultures ($P < 0.05$) contained non-*Campylobacter* colonies compared with the Bolton broth cultures. Significantly ($P < 0.05$) fewer quadrants from postchill carcass rinse samples, regardless of enrichment broth, contained non-*Campylobacter* colonies compared with rehang carcass rinse samples. The non-*Campylobacter* colonies most often observed on the Campy-cefex agar plates of the TECRA broth cultures were *Candida albicans* and *Escherichia coli.* The non-*Campylobacter* colonies most often observed on the Campy-cefex agar plates of the Bolton broth cultures were *Aeromonas sobria, Acinetobacter baumannii,* and *Pseudomonas fluorescens.*

DISCUSSION

In a poultry processing plant, samples taken at the rehang station usually contain larger populations of *Campylobacter* spp. and other organisms than do samples taken down the processing line where antimicrobial interventions have occurred such as exposure to chlorine in the chill tank *(5). Campylobacter* counts typically decrease in the scalding tank and increase during removal of feathers (picking) *(7).* In most commercial processing facilities in the United States, carcasses are chilled by immersion in air-agitated ice water in a series of tanks *(5, 7).* Examination of carcass rinsates at rehang and postchill allowed for the methods in the current study to be evaluated for *Campylobacter* recovery from samples in which a high number of competitive organisms are present or injured or stressed cells may be present. Bolton broth was employed as the standard broth for comparison because of previous research findings and its wide use in poultry research for recovery of *Campylobacter* spp. *(34, 39).* Several enrichment broths have been evaluated for *Campylobacter* recovery *(4, 34, 40).* In a comparison of Bolton broth, Preston broth, and *Campylobacter* selective broth (CEB), CEB and Bolton broth cultures had more *Campylobacter* spp. growth than did Preston broth cultures of various food matrices *(4).* In another study *(40),* Bolton broth was more sensitive than Preston broth. In the current study, TECRA broth outperformed both Bolton broth and direct plating for recovery of *Campylobacter* spp. from rinsate.

The four-quadrant streak method, which is a modification of an ecometric technique *(32),* was used to evaluate the presence of non-*Campylobacter* organisms on Campy-cefex agar and provided a means to compare the efficacy of these two broths for suppressing non-*Campylobacter* microflora during enrichment. This method provided a semiquantitative evaluation of non-*Campylobacter* microflora present in the broth cultures and a quick determination of the general ability of each broth to suppress the growth of non-*Campylobacter* organisms during enrichment. The organisms contaminating the plates in this study have been isolated in other studies *(12, 24).* Competitive organisms were suppressed more often in TECRA broth than in Bolton broth. The lack of non-*Campylobacter* organism suppression in Bolton broth allowed for overgrowth on the plating medium. This overgrowth might explain why fewer carcasses were determined to be *Campylobacter* spp. positive in Bolton broth cultures of rehang samples.

The difference in the suppression of competitive organisms and the type of organisms most often associated with the two broths could have been due to the antibiotics used in the two broths. The supplement used in Bolton broth to combat overgrowth contained cefoperazone, vancomycin, trimethoprim, and cycloheximide. The supplement for TECRA broth contained trimethoprim, rifampin, and polymyxin B. The differences in selective supplements most likely contributed to the differences in which competitive organisms were observed on the Campy-cefex plates. Most antibiotics used in *Campylobacter* enrichment broths are not adequate for reducing the level of competitive organisms when these organisms are present in samples in high numbers *(13, 29).*

An aerobic incubation environment was used for the TECRA protocol, and a microaerobic environment was used for the Bolton broth protocol. Because diverse organisms were present in the rinsates, the incubation environment could have influenced the growth of background microflora. Because of the diverse background microflora within the rinsate, further studies are needed to better assess this assumption. Blood (Bolton) and ferrous sulfate (TECRA) were the oxygen scavengers that contributed to a favorable growth environment for *Campylobacter.* In the past, Bolton broth cultures have been incubated in an aerobic environment, but studies have shown that a microaerobic environment increases the recovery of injured or stressed *Campylobacter* cells *(13).* Additional studies evaluating the incubation environment of TECRA broth may determine whether the sensitivity can be increased by microaerobic incubation.

TECRA broth was more efficient for suppressing competitive organisms and recovering *Campylobacter* spp. from rehang carcasses than was direct plating onto Campy-cefex agar plates. Direct plating was insufficient for evaluating the prevalence of *Campylobacter* spp. on postchill carcass-

es, providing a prevalence of only 19%. The low prevalence of *Campylobacter* spp. on carcasses as determined by direct plating has been documented in other studies *(5, 45)*. The chilling process, which includes application of antimicrobials, may injure or destroy *Campylobacter* cells, as was evident in the present study. The minimum detectable level by direct plating was presumed to be 1 CFU/ml, but the prevalence of *Campylobacter* spp. in postchill carcasses was significantly higher ($P < 0.05$) after enrichment than for direct plating. Carcasses sampled postchill may have contained sublethally injured cells. The data from Tables 3 and 4, which show the prevalence as determined by evaluating combinations of methods, suggest that increases in *Campylobacter* recovery in carcass rinse samples can be achieved by using more than one method of analysis.

The prevalences of *Campylobacter* spp. in postchill broiler carcasses were 71, 74, and 84% based on cultures grown in Bolton broth, TECRA broth, or both broths, respectively, but was only 19% based on results of direct plating of rinse samples. The methodology employed can affect the accuracy of *Campylobacter* spp. prevalence determined from carcasses at the final stages of processing. Several studies have been conducted to better understand the survival mechanisms used by *Campylobacter* spp. *(19, 26, 31, 38)*. The ability of injured or stressed *Campylobacter* cells to recover in environments outside the animal host and the significance of this ability for food safety are not fully understood *(37)*. Even stressed or sublethally injured cells present on a carcass must be considered in prevalence studies. The differences in procedures employed in the present study are representative of the various approaches used to recover *Campylobacter* spp. Direct plating allows for the evaluation of a processing step for reducing the level of contamination on a carcass, whereas this type of evaluation is not possible when an enrichment step is used *(5)*. However, the direct plating method did not accurately assess the prevalence of *Campylobacter* spp. on carcasses after the chilling process. Results from this study indicate that use of the TECRA broth method increased the number of poultry carcass rinsates determined to be *Campylobacter* positive compared with the other two methods. Processing steps can influence the sensitivity of different methods, and the combination of multiple methods increased the overall accuracy of *Campylobacter* prevalence results.

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REFERENCES

1. Anonymous. 2006. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, United States 2005. *Morb. Mortal. Wkly. Rep.* 55:392–395.
2. Bailey, J. S., P. Fedorka-Cray, L. J. Richardson, N. A. Cox, and J. M. Cox. 2008. Detection of *Campylobacter* from broiler carcass rinse samples utilizing the TECRA® visual immunoassay (VIA). *J. Rapid Methods Automation Microbiol.* 16:374–380.
3. Basa, S., A. Pal, and P. K. Desai. 2005. Quality control of culture media in a microbiology laboratory. *Indian J. Med. Microbiol.* 23: 159–163.
4. Baylis, C. L., S. MacPhee, K. W. Martin, T. J. Humphrey, and R. P. Betts. 2000. Comparison of three enrichment media for the isolation of *Campylobacter* spp. from foods. *J. Appl. Microbiol.* 89:884–891.
5. Berrang, M. E., J. S. Bailey, S. F. Altekruse, B. Patel, W. K. Shaw, Jr., R. J. Meinersmann, and P. J. Fedorka-Cray. 2007. Prevalence and numbers of *Campylobacter* on broiler carcasses collected at rehang and postchill in 20 U.S. processing plants. *J. Food Prot.* 70:1556–1560.
6. Berrang, M. E., R. J. Buhr, J. A. Cason, and J. A. Dickson. 2001. Broiler carcass contamination with *Campylobacter* from feces during defeathering. *J. Food Prot.* 64:2063–2066.
7. Berrang, M. E., and J. A. Dickens. 2000. Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant. *J. Appl. Poult. Res.* 9:43–47.
8. Berrang, M. E., J. K. Northcutt, and J. A. Cason. 2004. Recovery of *Campylobacter* from broiler feces during extended storage of transport cages. *Poult. Sci.* 83:1213–1217.
9. Beuchat, L. R. 1985. Efficacy of media and methods for detecting and enumerating *Campylobacter jejuni* in refrigerated chicken meat. *Appl. Environ. Microbiol.* 50:934–939.
10. Black, R. E., M. M. Levine, M. L. Clements, T. P. Hughes, and M. J. Blaser. 1988. Experimental *Campylobacter jejuni* infection in humans. *J. Infect. Dis.* 157:472–479.
11. Blaser, M. J., J. L. Penner, and J. G. Wells. 1982. Diversity of serotypes involved in outbreaks of *Campylobacter* enteritis. *J. Infect. Dis.* 146:825–829.
12. Bolton, F. J., and D. Coates. 1983. Development of a blood-free *Campylobacter* medium: screening tests on basal media and supplements, and the ability of selected supplements to facilitate aerotolerance. *J. Appl. Bacteriol.* 54:115–125.
13. Corry, J. E. L., D. E. Post, P. Colin, and M. J. Laisney. 1995. Culture media for the isolation of campylobacters. *Int. J. Food Microbiol.* 26:43–76.
14. Cox, N. A., J. E. Thomson, and J. S. Bailey. 1983. Procedure for isolation and identification of *Salmonella* from poultry carcasses. Agriculture handbook 603. U.S. Department of Agriculture, Agricultural Research Service, Washington, DC.
15. Dunn, O. J. 1964. Multiple contrasts using rank sums. *Technometrics* 5:241–252.
16. Friedman, C. R., J. Neimann, H. C. Wegener, and R. V. Tauxe. 2000. Epidemiology of *C. jejuni* infections in the United States and other industrialized nations, p. 121–138. *In* I. Nachamkin and M. J. Blaser (ed.), *Campylobacter*, 2nd ed. ASM Press, Washington, DC.
17. Gormley, F. J., M. MacRae, K. J. Forbes, I. D. Osden, J. F. Dallas, and N. J. C. Strachan. 2008. Has retail chicken played a role in the decline of human campylobacteriosis? *Appl. Environ. Microbiol.* 74: 383–390.
18. Habib, I., I. Sampers, M. Uyttendaele, D. Berkvens, and L. DeZutter. 2008. Performance characteristics and estimation of measurement uncertainty of three plating procedures for *Campylobacter* enumeration in chicken meat. *Food Microbiol.* 25:65–74.
19. Hazeleger, W. C., J. A. Wouters, F. M. Rombouts, and T. Abee. 1998. Physiological activity of *Campylobacter jejuni* far below the minimal growth temperature. *Appl. Environ. Microbiol.* 64:3917–3922.
20. Hook, H., M. A. Fattah, H. Ericsson, I. Vagsholm, and M. L. Danielsson-Tham. 2005. Genotype dynamics of *Campylobacter jejuni* in a broiler flock. *Vet. Microbiol.* 106:109–117.
21. Humphrey, T. J. 1989. An appraisal of the efficacy of pre-enrichment for the isolation of *Campylobacter jejuni* from water and food. *J. Appl. Bacteriol.* 66:119–126.
22. Jacobs-Reitsma, W. 2000. *Campylobacter* in the food supply, p. 467–481. *In* I. Nachamkin and M. J. Blaser (ed.), *Campylobacter*, 2nd ed. ASM Press, Washington, DC.
23. Jeffrey, J. S., A. Hunter, and E. R. Atwill. 2000. A field-suitable, semisolid aerobic enrichment medium for isolation of *Campylobacter jejuni* in small numbers. *J. Clin. Microbiol.* 38:1668–1669.
24. Karmali, M. A., A. E. Simor, M. Roscoe, P. C. Fleming, S. S. Smith, and J. Lane. 1986. Evaluation of blood-free, charcoal-based selective medium for the isolation of *Campylobacter* organisms from feces. *J. Clin. Microbiol.* 23:456–459.
25. Keener, K. M., M. P. Bashor, P. A. Curtis, B. W. Sheldon, and S.

Kathariou. 2004. Comprehensive review of *Campylobacter* and poultry processing. *Compr. Rev. Food Sci. Food Saf.* 3:105–116.

26. Kelly, A. F., S. F. Park, R. Bovill, and B. M. Mackey. 2001. Survival of *Campylobacter jejuni* during stationary phase: evidence for the absence of a phenotypic stationary-phase response. *Appl. Environ. Microbiol.* 67:2248–2254.
27. Kornacki, J. L., J. B. Gurtler, Z. Yan, and C. M. Cooper. 2003. Evaluation of several modifications of an ecometric technique for assessment of media performance. *J. Food Prot.* 66:1727–1732.
28. Lastovica, A. J. 2006. Emerging *Campylobacter* spp.: the tip of the iceberg. *Clin. Microbiol. Newsl.* 28(7):49–56.
29. Line, E. J. 2001. Development of a selective differential agar for isolation and enumeration of *Campylobacter* spp. *J. Food Prot.* 11: 1711–1715.
30. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607–625.
31. Moen, B., A. Oust, Ø. Langsrud, N. Dorrell, G. L. Marsden, J. Hinds, A. Kohler, B. W. Wren, and K. Rudi. 2005. Explorative multifactor approach for investigating global survival mechanisms of *Campylobacter jejuni* under environmental conditions. *Appl. Environ. Microbiol.* 71:2086–2094.
32. Mossel, D. A. A., F. van Rossem, M. Koopmans, M. Hendriks, M. Verouden, and I. Eelderink. 1980. Quality control of solid culture media: a comparison of the classic and the so-called ecometric technique. *J. Appl. Bacteriol.* 49:439–454.
33. Northcutt, J. K., M. E. Berrang, J. A. Dickens, D. L. Fletcher, and N. A. Cox. 2005. Effect of broiler age, feed withdrawal, and transportation on levels of coliforms, *Campylobacter, Escherichia coli* and *Salmonella* on carcasses before and after slaughter. *Poult. Sci.* 82:169–173.
34. Oyarzabal, O. A., S. Backert, M. Nagaraj, R. S. Miller, S. K. Hussain, and E. A. Oyarzabal. 2007. Efficacy of supplemented buffered peptone water for the isolation of *Campylobacter jejuni* and *C. coli* from broiler retail products. *J. Microbiol. Methods* 69:129–136.
35. Oyarzabal, O. A., K. S. Macklin, J. M. Barbaree, and R. S. Miller. 2005. Evaluation of agar plates for direct enumeration of *Campylobacter* spp. from poultry carcass rinses. *Appl. Environ. Microbiol.* 71:3351–3354.
36. Park, C. E., Z. K. Stankiewicz, J. Lovett, J. Hunt, and D. W. Francis. 1983. Effect of temperature, duration of incubation, and pH on enrichment culture on the recovery of *Campylobacter jejuni* from eviscerated market chickens. *Can. J. Microbiol.* 29:803–806.
37. Park, S. F. 2002. The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. *Int. J. Food Microbiol.* 74:177–188.
38. Parkhill, J., B. W. Wren, K. Mungall, J. M. Ketley, C. Churcher, D. Basham, T. Chillingworth, R. M. Davies, T. Feltwell, S. Holroyd, K. Jagels, A. V. Karlyshev, S. Moule, M. J. Pallen, C. W. Penn, M. A. Quail, M. A. Rajandream, K. M. Rutherford, A. H. van Vliet, S. Whitehead, and B. G. Barrell. 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature* 403:665–668.
39. Paulsen, P., P. Kanzler, F. Hilbert, S. Mayrhofer, S. Baumgartner, and F. J. M. Smulders. 2005. Comparison of three methods for detecting *Campylobacter* spp. in chilled or frozen meat. *Int. J. Food Microbiol.* 103:229–233.
40. Potturi-Venkata, L. P., S. Brackert, A. J. Lastovica, S. L. Vieira, R. A. Norton, R. S. Miller, S. Pierce, and O. A. Oyarzabal. 2007. Evaluation of different plate media for direct cultivation of *Campylobacter* species from live broilers. *Poult. Sci.* 86:1304–1311.
41. Rosenquist, H., A. Bengtsson, and T. B. Hansen. 2007. A collaborative study on a Nordic standard protocol for detection and enumeration of thermotolerant *Campylobacter* in food. *Int. J. Food Microbiol.* 118:201–213.
42. Rosenquist, H., H. M. Sommer, N. L. Nielson, and B. B. Christensen. 2006. The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter. Int. J. Food Microbiol.* 108:226–232.
43. Shimada, K., and H. Tsuji. 1986. Enrichment for detection of *Campylobacter jejuni. J. Clin. Microbiol.* 23:887–890.
44. Skirrow, M. B. 1991. Epidemiology of *Campylobacter* enteritis. *Int. J. Food Microbiol.* 12:9–16.
45. Stern, N. J., and S. Pretanik. 2006. Counts of *Campylobacter* spp. on U.S. broiler carcasses. *J. Food Prot.* 69:1034–1039.